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FINAL REPORT

IS HEPATITIS E VIRUS A COFACTOR IN THE DEVELOPMENT OF POST WEANING MULTISYSTEMIC WASTING SYNDROME IN PIGS

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Final Report to the Saskatchewan Agriculture Development Fund for the Research Project Titlted:

"Is Hepatitis E Virus a Cofactor in the Development of Post Weaning Multisystemic Wasting Syndrome in Pigs?"

JA Ellis

Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan

Abstract / Summary

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The overall objective of the proposed studies was to determine the prevalence of the newly described porcine hepatitis E virus (HEV), and to determine if this virus plays a pathogenic role in pigs affected with post weaning multisystemic syndrome. Tissues of pigs with PMWS were examined for the presence HEV sequences using polymerase chain reaction (PCR). A 344 bp fragment was amplified using reverse-transcriptase-polymerase chain reaction from the tissues of pigs with of only PMWS. Antibodies to HEV were found in clinically normal and diseased pigs. Results of these analyses suggest that HEV is not a primary pathogen in PMWS, but cannot rule the possibility that it could be a cofactor in the disease expression.

Executive Summary

Postweaning multisystemic wasting syndrome (PMWS), is a new disease that most often affects 5-12 week old pigs, and has been recognized in swine

herds in Canada, the United States, and Europe. Clinically, the disease is characterized by progressive weight loss, tachypnea, dyspnea, and jaundice. Lesions in affected pigs include lymphadenopathy, lymphocytic to granulomatous interstitial pneumonia, lymphocytic to granulomatous hepatitis and nephritis.

We have recently documented a high degree of association between the presence of a circovirus-like virus and PMWS in affected swine (Ellis et al., 1998). With the support of our industrial partner, studies confirmed the role of circoviruses in PMWS. However, based on recent publications (reviewed in Meng et al., 1997) and our preliminary data, it was critical to determine if the newly described porcine hepatitis plays a role PMWS. This virus could be an important pathogen in the syndrome, and also an agent with significance to public health.

The objectives of this study were twofold. Firstly to screen for HEV in pigs with PMWS using a sensitive assay to detect viral RNA and, secondly, to determine of seroconversion to HEV was associated with PMWS.

In order to investigate the possible role of HEV in PMWS, various tissues were examined for the presence of HEV using a reverse transcriptase polymerase chain reaction. A 344 bp fragment was amplified from tissues from only 2 of 35 pigs with PMWS. An ELISA was used to detect antibodies to HEV in diseased and normal pigs. Antibodies to HEV were found in high prevalence in both groups of pigs. Together the results of these analyses suggest that HEV is not a primary pathogen in PMWS.

Technical Report

METHODS

I. SOURCE OF PORCINE TISSUES AND CELL LINES

Tissues, including, blood, liver, lung, kidney, lymphoid nodes and bone marrow were collected from debilitated 5-12 week old pigs exhibiting clinical signs and multisystemic lesions that are characteristic of PMWS. Identical tissues

were collected from similarly aged, clinically normal control pigs from a farm which has not had a case of PMWS. In addition, porcine circovirus-tested porcine kidney (PK-15) cells and Madin-Darby bovine kidney (MDBK) cell lines were also used as negative controls.

II. DETECTION OF HEV RNA REVERSE TRANSCRIPTASE (RT) POLYMERASE CHAIN REACTION (RT-PCR):

RNA used as a substrate for the reverse transcriptase-polymerase chain reaction (RT-PCR) was be obtained from livers of pigs with PMWS (and appropriate controls) using standard techniques. RT-PCR will be used to attempt to amplify conserved hepatitis E viral sequences using degenerate and nested primers specific for a conserved region of the capsid gene (Meng et al., 1997) These primers had been previously used to successfully amplify most known human and porcine hepatitis E viruses (Meng et al., 1997). PCR products of the expected size were hybridized with a specific probe (an amplified conserved fragment common to known hepatitis E viruses) by standard Southern blot techniques.

When specificity is confirmed by positive hybridization with the probe, fragments will be eluted and cloned into a suitable vector (pCRII, Invitrogen). Positive clones will be identified using the same probe as above by Southern blotting.

Clones containing HEV fragments were sequenced by the double strand protocol (Double Strand Sequencing Kit, BRL Laboratories) and sequences will be compared with other Hepatitis E viruses contained in the GenBank. Positive controls in these assays included RNA from human Hepatitis E virus (capsid gene). Negative controls included examination of RNA derived from cell lines of porcine origin, such as porcine kidney and testicular cells; cells lines of non-porcine origin, such as Madin-Darby bovine kidney cells; tissue from gnotobiotic

pigs; tissue from gnotobiotic pigs and pigs from farms on which PMWS has never been diagnosed.

III. DETECTION OF ANTIBODIES TO HEV BY ELISA:

An ELISA for Hepatitis E virus-specific antibodies will be performed as previously described (Meng et al., 1997). Positive controls in these assays will include hyerimmune pig serum (Meng et al., 1997). Negative controls will include normal sera from gnotobiotic pigs.

RESULTS

I. **Identification of Hepatitis E viral sequences in livers from pigs with PMWS.** Using the degenerate and nested RT-PCR primers as indicated, an approximately 344 bp fragment in RNA was amplified from liver samples from 2 of 35 pigs with PMWS (Figure 1). All of these pigs had high concentrations of porcine circovirus 2 in one or more organs. This fragment was not amplified from the livers of normal pigs, nor from cultured PK-15 cells. The identity of this fragment was confirmed by sequencing.

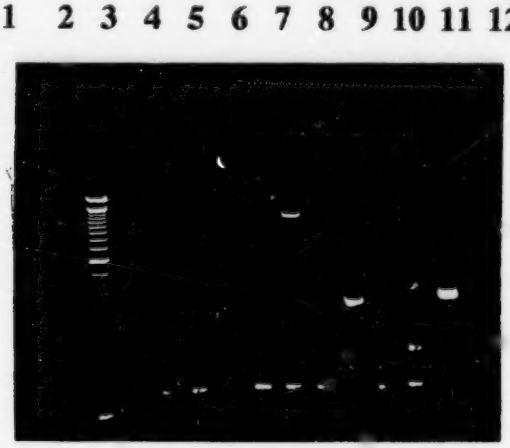

Figure 1. RT-PCR for conserved Hepatitis E viral sequences using RNA extracted from tissues 10 of pigs with PMWS (lanes 2- 11) Note 344 bp fragment in RNA from 1 PMWS pig (lane 9). Lane 1 is DNA ladder; lane 12 is positive control

II. **Detection of antibodies to HEV in pigs.** Antibodies to HEV were found in various ages of the serum of pigs with PMWS, and clinically normal pigs, with no apparent differences between group.

DISCUSSION

The results of this study indicate an apparent low prevalence of active infection with HEV in pigs with PMWS, all of which had evidence of active infection with porcine circovirus 2. As in previous studies (Meng et al., 1997) antibodies to HEV were found in pigs with no apparent correlation with clinical status, normal versus diseased. Taken together these results suggest that HEV is not a primary or necessary etiologic agent in PMWS. Whether or not infection by HEV plays a role as a co-factor, as is the case with porcine parvovirus (Ellis et al., 2000), in the pathogenesis of PMWS is unresolved. Further prospective studies are necessary to resolve this issue.

References

Allan GM and Ellis JA. (2000). Porcine circoviruses: A review. J Vet Diagn Invest 12:3-14.

Ellis JA, et al. (2000). Co-infection by porcine circovirues and porcine parvovirus in pigs with naturally acquired post weaning multisystemic wasting syndrome. J Vet Diagn Invest 12:21-27.

Meng XJ, et al. (1997). A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA; 94:9860-9865.

Project Activities, Achievements, Papers, Presentations

A manuscript based on these data is in preparation for submission to the *Canadian Veterinary Journal.* As well these data have been presented or referred to in several intramural and extramural seminars, including the annual meeting of the

American College of Veterinary Pathologists. These forums were efficient means of transmission of these findings.

Indications for Further Technical or Commerical Development

These data indicate no need at present to test commericial pigs for HEV.

Information of Benefit to Producer's, Government or Industry

These data indicate the recently described HEV is apparently not involved as a primary pathogen in PMWS. The role of this virus in causing other clinical disease in pigs is still under investigation

Personnel

Dr. Ana Bratanich was employed as a post-doctoral fellow during this project, and Carrie Konoby (Technician level II) was employed throughout the duration of the project.

Project Developed Materials

None produced or anticipated.

SADF Acknowledgement

The support of SADF has been acknowledged in a recently published review article on porcine circoviruses, as well as in several seminars related to PMWS.